ELSEVIER

Pharmacology, Biochemistry and Behavior 71 (2002) 245–249

PHARMACOLOGY BIOCHEMISTRY AND BEHAVIOR

www.elsevier.com/locate/pharmbiochembeh

Protective effect of *trans*-resveratrol against kainic acid-induced seizures and oxidative stress in rats

Y.K. Gupta*, Seema Briyal, Geeta Chaudhary

Department of Pharmacology, Neuropharmacology Laboratory, All India Institute of Medical Sciences, New Delhi 110029, India

Received 6 February 2001; received in revised form 21 August 2001; accepted 11 September 2001

Abstract

Overexcitation of excitatory amino acid is an important mechanism in seizure genesis wherein free radicals have recently been suggested to play a critical role. Thus, intervention by antioxidants can be a potential beneficial approach in the treatment of epilepsy. The present study was undertaken to see the effect of *trans*-resveratrol, a potent antioxidant, against kainic acid-induced seizures, and effect on markers of oxidative stress in brain. Kainic acid, 10 mg/kg ip, induced long-lasting seizures and associated symptoms. The brain level of malondialdehyde (MDA) was found to be significantly raised after kainic acid administration (295 ± 18 nmol/g wet tissue) as compared to control (195 ± 26 nmol/g wet tissue). Pretreatment (5 min) of single dose of *trans*-resveratrol (40 mg/kg ip) could not inhibit the convulsions though the latency was significantly increased. When multiple doses of *trans*-resveratrol were injected in two-dose schedules in different animals (20 and 40 mg/kg ip, 5 min prior and repeated 30 and 90 min after kainic acid), there was significant reduction in incidence of convulsions in both treatment schedules. The brain MDA levels were found to be significantly attenuated in the *trans*-resveratrol-treated groups (multiple doses of 20 and 40 mg/kg) as compared to the kainic acid alone. However, the glutathione level in control, kainic acid- and *trans*-resveratrol-treated animals were not significantly different. The protective effect of *trans*-resveratrol against kainic acid-induced convulsions and the attenuation of raised MDA level suggest the potential use of antioxidants at least as adjunct therapy in epilepsy. © 2002 Elsevier Science Inc. All rights reserved.

Keywords: Kainic acid; Seizures; Antioxidants; *Trans*-resveratrol; Rats

1. Introduction

Epilepsy is the most common neurological disorder worldwide. Various mechanisms for the genesis of seizures have been proposed; however, overexcitation of excitatory amino acid and inhibition of GABAergic system have gained much acceptance. (Engelborghs et al., 2000; Friedman et al., 1994; Heinemann et al., 1994).

Recently, it has been proposed that activation of excitatory amino acid receptor can trigger the formation of reactive oxygen species (ROS). These increased ROS, in turn, may further release glutamate, thus forming a loop. This 'vicious' cycle not only causes long-lasting seizure formation, but if not arrested may lead to neuronal death (Atlante et al., 2001; Bondy, 1995; Coyle and Puttfarcken, 1993; Nakao and Brundin, 1998; Said et al., 2000). Status epilepticus is such an emergency condition where seizures last for a long time and, if not controlled, neuronal injury occurs (Cherlyee and Thomos, 1995). Several experimental models of status epilepticus have been developed like lithium–pilocarpine model, kainic acid-induced model, etc. (Ben, 1985; Chaudhary et al., 1999; Jope et al., 1986). Kainic acid is an analogue of glutamic acid and when injected systemically or intracerebrally in animals produce convulsions by activation of the excitatory amino acid receptors (Ben, 1985). In various experimental studies, it has been demonstrated that antioxidants can prevent the excitotoxicity induced by agents like glutamate and domoic acid (Cazevieille et al., 1997; Kim et al., 2000; Lancelot et al., 1997; Nakao et al., 1996; Teismann and Ferger, 2000). Thus, the antioxidants may have a potential role in preventing excitotoxicity-induced seizure genesis.

After the realization of reduced cardiac risk in the consumers of red wine, popularly referred as French paradox, much interest has emerged in resveratrol, which is the active constituent of red wine (Fermont, 2000). Resveratrol

* Corresponding author. Tel.: +91-659-3684; fax: +91-686-4789.
E-mail address: ykg@hotmail.com (Y.K. Gupta).

PII: S0091-3057(01)00663-3

(3,4,5-tri-hydroxy stilbene) is a naturally occurring phytoalexin present in high concentration in skin of grapes and in wine. It exists in *cis* and *trans* isomeric form and the concentration of *trans* isomer, the major form, contributes to its biological activity (Fermont, 2000).

Trans-resveratrol has been shown to have a potent free radical scavenging activity in various in vitro and in vivo studies. In the rat liver microsomes, *trans*-resveratrol inhibited the metal-induced lipid peroxidation (Chanbitayapongs et al., 1997) and also prevented lipid peroxidation in PC12 cells induced by iron and ethanol (Sun et al., 1997). It has also shown a cardioprotective action against ischemia perfusion injury in isolated rat heart and this activity was proposed to be due to its antioxidant property (Ray et al., 1999).

The present study was therefore designed to evaluate the effect of *trans*-resveratrol against kainic acid-induced status epilepticus.

2. Materials and methods

2.1. Animals

Albino Wistar rats of either sex weighing 200–250 g were used for the study. The animals were procured from the central animal facility at All India Institute of Medical Sciences, New Delhi. The rats were group housed in polypropylene cages ($38 \times 23 \times 10$ cm) with not more than six animals per cage. They were maintained under standard laboratory conditions with natural dark–light cycle and were allowed free access to standard dry rat diet (Golden Feeds, India) and tap water ad libitum. The experimental groups contained seven animals each. All experimental procedures in rats described were reviewed and approved by the Institutional Animal Ethics Committee.

2.2. Drugs

Kainic acid (Sigma, St. Louis, MO, USA) was dissolved in distilled water. *Trans*-resveratrol (Courtesy; Pharmascience, Montreal, Canada) of strength 2% was prepared freshly by dissolving in 50% alcohol. Thus, in administrating the dose of 20 and 40 mg/kg ip in the rats ranging from 200 to 250 g, the total volume injected intraperitoneally ranged between 0.2 and 0.5 ml, which was below the recommended limit of 1 ml/100 g body weight in the rats.

2.3. Experimental seizure model

Rats were administered kainic acid at a dose of 10 mg/kg ip, pH adjusted to 7.2 ± 0.1. Animals were than observed for behavioral changes (grooming, rearing, hind limb scratching, urination, defecation, wet dog shakes, jaw movements, salivation, head nodding), incidence and latency of convulsions and mortality over the total period of 4 h. The rats were thereafter sacrificed for estimation of malondialdehyde (MDA) and glutathione markers of oxidative stress.

2.4. Experimental protocol and drug

Rats were divided into four groups. In one group, *trans*-resveratrol was injected at the dose of 40 mg/kg ip 5 min prior to kainic acid administration and observed over a period of 4 h for the change in behavioral parameters, incidence and latency of convulsions and the mortality rate.

In the second and third groups, *trans*-resveratrol was injected at the dose of 20 and 40 mg/kg ip 5 min prior to kainic acid administration, respectively. The doses, 20 and 40 mg/kg, were again repeated 30 and 90 min after kainic acid (total *trans*-resveratrol administered being 60 and 120 mg/kg, respectively) and observed over a period of 4 h for all the parameters described above. The fourth group was the vehicle-treated control group ($n = 7$) and the rats were administered 50% ethanol in the volume and the dose schedule followed for the administration of multiple doses of *trans*-resveratrol.

The rats in all the groups were sacrificed under chloroform anesthesia after 4 h for the estimation of brain MDA and glutathione level. The brains were quickly removed, cleaned with chilled saline and stored at -70 °C until biochemical analysis, which was carried out within 2 days.

2.5. Measurement of lipid peroxidation

MDA, which is a measure of lipid peroxidation, was measured spectrophotometrically (Okhawa et al., 1979). Briefly, brain tissues were homogenized with 10 times (w/v) 0.1 sodium phosphate buffer (pH 7.4). The reagents acetic acid 1.5 ml (20%), pH 3.5, 1.5 ml of thiobarbituric acid (0.8%) and 0.2 ml of sodium dodecyl sulfate (8.1%) were added to 0.1 ml of processed tissue sample. The mixture was then heated at 100 °C for 60 min. The mixture was cooled with tap water and 5 ml of *n*-butanol:pyridine (15:1% v/v); 1 ml of distilled water was added. The mixture was shaken vigorously. After centrifugation at 4000 rpm for 10 min, the organic layer was withdrawn and absorbance was measured at 532 nm using a spectrophotometer.

2.6. Measurement of glutathione

Glutathione was measured spectrophotometrically (Ellman, 1959). Briefly, brain tissues were homogenized with 10 times (w/v) 0.1 M sodium phosphate buffer (pH 7.4). This homogenate was then centrifuged with 5% trichloroacetic acid to centrifuge out the proteins. To 0.1 ml of this homogenate, 2 ml of phosphate buffer (pH 8.4), 0.5 ml of 5′5 dithiobis (2-nitrobenzoic acid) (DTNB) and 0.4 ml of double distilled water were added. The mixture was vortexed and the absorbance read at 412 nm within 15 min.

2.7. Statistical analysis

The data are represented as mean ± S.E.M. The unpaired Student's *t* test is used for statistical analysis.

3. Results

3.1. Effect of kainic acid on behavioral symptoms and convulsions

Kainic acid, when administered per se at the dose of 10 mg/kg ip, exhibited behavioral signs, i.e., grooming, rearing, hind limb scratching, urination, defecation, wet dog shakes, jaw movements, salivation, head nodding in all the rats within 5 min. One hundred percent of the rats exhibited convulsions with the mean latency of 53 ± 10.2 min. The percent incidence in the vehicle-treated kainic acid group was 100% and the mean latency of convulsions was 61 ± 14.0 min.

3.2. Effect of trans-resveratrol on kainic acid-induced seizures

When *trans*-resveratrol was administered at the dose of 40 mg/kg ip 5 min before the administration of kainic acid (10 mg/kg ip), all the animals exhibited behavioral signs. However, the latency of occurrence of behavioral signs increased to 30 min as compared to 5 min in kainic acid per se group. All the animals in the group showed convulsions though the latency was significantly increased (121.7 ± 9.4 min) as compared to that in vehicle-treated kainic acid group (61 ± 14.0 min) ($P < .05$).

In another set of experiment, *trans*-resveratrol was administrated at the dose of 20 mg/kg ip 5 min prior and repeated 30 and 90 min after kainic acid administration. There was a significant decrease in the percent incidence of forelimb clonus (42.8%) as compared to 100% in the vehicle-treated kainic acid group ($P < .05$). Four of seven rats showed behavioral symptoms, which started within 5 min of kainic acid administration but subsided after the second and third doses of *trans*-resveratrol.

In another group of experiment, *trans*-resveratrol was administered at the dose of 40 mg/kg ip 5 min prior to kainic acid and repeated 30 and 90 min after the administration. There was a significant decrease in the percent incidence of forelimb clonus (14%) as compared to that in vehicle-treated kainic acid group (100%) ($P < .05$). In this group, the mean latency increased to 186.0 ± 60.7 min as compared to that in vehicle-treated kainic acid group (61 ± 14.0 min). In this group, no behavioral symptoms were observed in six of seven nonconvulsive rats as that seen in which the multiple doses of *trans*-resveratrol (20 mg/kg ip) were administered (Fig. 1).

3.3. Effect of kainic acid, 50% ethanol (vehicle) and trans-resveratrol on brain MDA level

The brain levels of MDA were found to be significantly raised after kainic acid administration (295 ± 18 nmol/g wet tissue) as compared to the control rats (195 ± 26 nmol/g wet tissue) ($P < .05$). The levels of MDA were also found to be significantly increased in the vehicle-treated kainic acid group — the value being 293 ± 42 nmol/g wet tissue. The levels of MDA were insignificant in the vehicle-treated group as compared to that in kainic acid per se group.

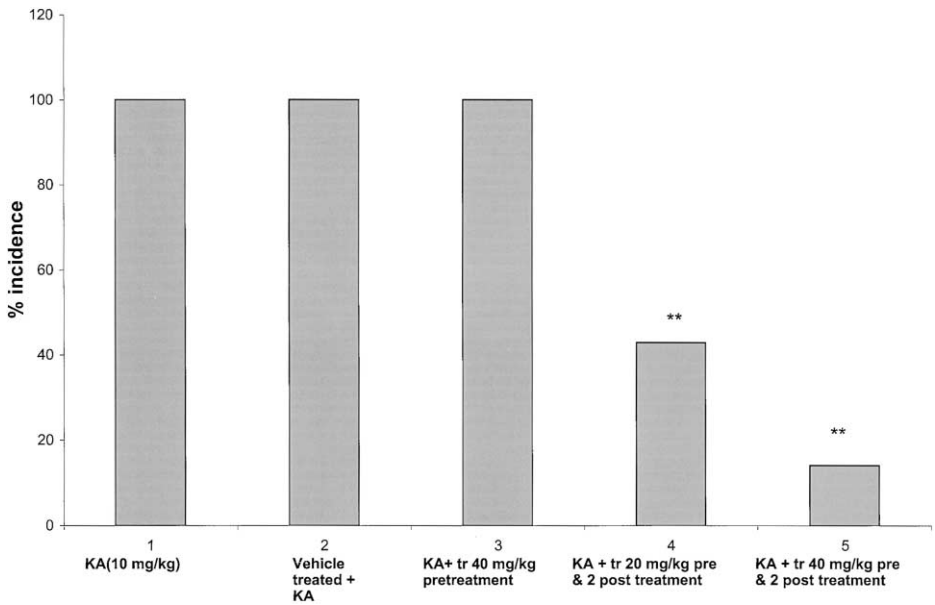

Fig. 1. Effect of *trans*-resveratrol on percent incidence of convulsions induced by kainic acid in rats. ** $P < .05$ vs. vehicle-treated kainic acid group. 'KA' represents 'kainic acid' and 'tr' represents '*trans*-resveratrol' in the figure.

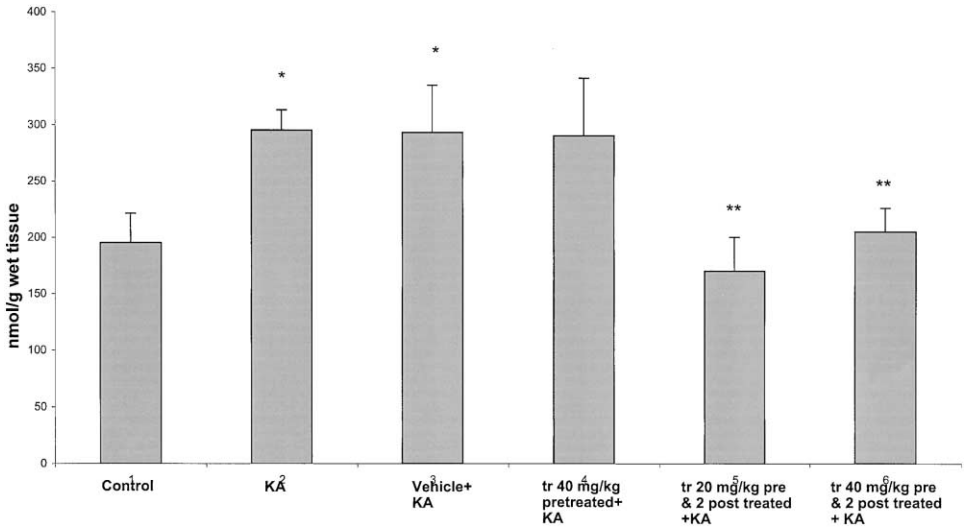

Fig. 2. Effect of *trans*-resveratrol on the levels of MDA in kainic acid-induced seizures in rats. Value expressed as nanomoles per gram wet tissue (mean ± S.E.M.). * $P < .05$ vs. control. ** $P < .05$ vs. vehicle-treated kainic acid group. 'KA' represents 'kainic acid' and 'tr' represents '*trans*-resveratrol' in the figure.

In the rats treated with *trans*-resveratrol (single dose of 40 mg/kg, 5 min pretreatment), there was insignificant change in the brain MDA levels (290 ± 51 nmol/g wet tissue) as compared to that in vehicle-treated kainic acid group (293 ± 42 nmol/g wet tissue).

In *trans*-resveratrol-treated group (20 mg/kg ip; multiple doses), the brain levels of MDA were found to be significantly lower after the administration of kainic acid (170 ± 30 nmol/g wet tissue) as compared to that in the vehicle-treated kainic acid group.

Similarly, in the group in which 40 mg/kg ip *trans*-resveratrol was administered in multiple doses, the brain levels of MDA were significantly attenuated (205 ± 21 nmol/g wet tissue) as compared to that in the vehicle-treated kainic acid group ($P < .05$). The effects of three doses of *trans*-resveratrol 20 mg/kg vs. three doses of *trans*-resveratrol 40 mg/kg were not significantly different (Fig. 2).

3.4. Effect of kainic acid, 50% ethanol (vehicle) and trans-resveratrol treatment on brain glutathione levels

The brain glutathione levels were estimated in control, kainic acid per se, vehicle-treated and *trans*-resveratrol-treated rats. The brain levels of glutathione showed insignificant change in kainic acid (88.7 ± 5 μg/g wet tissues) and vehicle-treated group as compared to the control rats (85 ± 5 μg/g wet tissues).

In the rats treated with *trans*-resveratrol (single dose of 40 mg/kg), there was insignificant change in the brain glutathione levels (93.3 ± 12 μg/g wet tissues) as compared to that in the vehicle-treated kainic acid group. Also in the multiple dose groups of 20 and 40 mg/kg ip, there was insignificant change in the brain glutathione levels — the values being 72.5 ± 5 and 74 ± 6 μg/g wet tissues, respectively.

4. Discussion

Overactivation of excitatory amino acid receptors is an important pathogenetic factor that leads to seizure genesis (Heinemann et al., 1994). Ample evidence from both in vivo and in vitro studies suggests that free radicals play a critical role in the enhancement of excitotoxicity (Scultz et al., 1995). Antioxidants like adenosine, melatonin, has shown to ameliorate the excitotoxicity (Lancelot et al., 1997; Nakao et al., 1996). Therefore, use of antioxidants could be a potential approach in arresting or inhibiting the seizure genesis caused by excitotoxic agents.

Trans-resveratrol, a polyphenolic compound, has shown potent antioxidant activity in various in vitro and in vivo studies (Cadenas and Barja, 1999; Ray et al., 1999; Sun et al., 1997). Kainic acid, an excitotoxin, causes consistent and long-lasting seizure in rats after systemic administration. In the present study, kainic acid 10 mg/kg ip produced behavioral changes as well as convulsions in all the animals. An increase in brain MDA level, a marker of oxidative stress, was also observed, suggesting the involvement of ROS in the kainic acid-induced convulsions. However, there was no change in the levels of glutathione. Glutathione is an essential tripeptide, and endogenous antioxidant found in all animal cells. It reacts with the free radicals and can protect from singlet oxygen, hydroxide radicals and superoxide radical damage (Shi et al., 1994). The reason for insignificant change in glutathione levels seen in our study could be because the enzymes involved in glutathione synthesis may maintain the levels of glutathione at least during the early stage of oxidative stress. Administration of *trans*-resveratrol single dose (40 mg/kg ip) did not inhibit the seizures; however, it significantly increased the latency of convulsions. Since *trans*-resveratrol has a short half-life of 30–45 min (Bertelli et al., 1996), whereas kainic acid

causes long-lasting seizures, we repeated the dose of *trans*-resveratrol twice after kainic acid administration to maintain its plasma level. The multiple doses of 20 and 40 mg/kg of *trans*-resveratrol resulted in the significant protection at both dose schedules.

This was well correlated with attenuation in the levels of MDA in the rats treated with multiple doses of *trans*-resveratrol, whereas no change was seen with single dose of *trans*-resveratrol.

Kainic acid is known to cause severe convulsions as a consequence to glutamate receptor stimulation. It is our contention that the animals will exhibit seizures when a threshold value of excitation is reached after administration of kainic acid. The kainic acid administration stimulates glutamate receptors, which enhances ROS level, which in turn will enhance glutaminergic activity. It is difficult to predict at what level *trans*-resveratrol acts. However, it is evident that it prevents the vicious chain, thus decreasing the excitotoxicity and thereby showing the protective effect. This suggests that the administration of CNS-selective antioxidants may have protective role in seizures occurring through this mechanism, and suggests its potential use in status epilepticus.

References

Atlante A, Calissano P, Bobba A, Giannattasios S, Morra E, Passarella S. Glutamate neurotoxicity, oxidative stress and mitochondria. FEBS Lett 2001;497:1–5.

Ben AY. Limbic seizure and brain damage produce by kainic acid: mechanisms and relevance to human temporal lobe epilepsy. Neuroscience 1985;14:375–403.

Bertelli AAE, Giovannini L, Stradi R, Urien S, Tillement JP, Bertelli A. Kinetics of *trans*- and *cis*-resveratrol (3,4′,5-trihydroxy stilbene) after red wine oral administration in rats. Int J Clin Pharmacol Res 1996; 16(4/5):77–81.

Bondy CS. The relation of oxidative stress and hyperexcitation to neurological disease. Proc Soc Exp Biol Med 1995;208:337–45.

Cadenas S, Barja G. Resveratrol, melatonin, vitamin E and PBN protect against renal oxidative DNA damage induced by the kidney carcinogen Kbro3. Free Radical Biol Med 1999;26:1531–7.

Cazevieille C, Safa R, Osborne NN. Melatonin protects primary cultures of rats cortical neurons from NMDA excitotoxicity and hypoxia/reoxygenation. Brain Res 1997;768:120–4.

Chanbitayapongs S, Draczynska LB, Sun AY. Amelioration of oxidative stress by antioxidant and resveratrol in PC12 cells. NeuroReport 1997; 8:1499–502.

Chaudhary G, Malhotra J, Gupta YK. Effect of different lithium priming schedules on pilocarpine induced status epilepticus in rats. Methods Find Exp Clin Pharmacol 1999;21:21–4.

Cherlyee CWJ, Thomos PB. Status epilepticus. Neurol Clin 1995;13: 529–48.

Coyle JT, Puttfarcken P. Oxidative stress, glutamate and neurodegenerative disorders. Science 1993;262:689–95.

Ellman GL. Tissue sulphyldryl groups. Arch Biochem Biophys 1959;82: 70–3.

Engelborghs S, D'Hooge R, De Deyn PP. Pathophysiology of epilepsy. Acta Neurol Belg 2000;100:201–13.

Fermont L. Biological effects of resveratrol. Life Sci 2000;66:663–73.

Friedman LK, Pellegrini GDE, Sperber EF, Bennett MV, Moshe SL, Zukin RS. Kainate-induced status epilepticus alters glutamate and GABA receptor gene expression in adult rat hippocampus: an in situ hybridization study. J Neurosci 1994;14(5 Pt 1):2697–707.

Heinemann U, Draguhu A, Ficker E, Stabel J, Zhang CL. Strategies for the development of drugs for pharmacoresistant epilepsies. Epilepsia 1994;35(Suppl. 5):S10–21.

Jope RS, Morriest RA, Snead DC. Characterization of lithium potentiation of pilocarpine-induced status epilepticus in rats. Exp Neurol 1986;91: 471–80.

Kim HC, Jhoo WK, Bing G, Shin EJ, Wie MB, Kim KH. Phenidone prevents kainate-induced neurotoxicity via antioxidant mechanisms. Brain Res 2000;874:15–23.

Lancelot E, Revaud ML, Boulu RG, Plotkine M, Callebert J. Alpha-phenly-*N*-*tert*-butylnitrone attenuates excitotoxicity in rat striatum by preventing hydroxyl radical accumulation. Free Radical Biol Med 1997;23(7): 1031–4.

Nakao N, Brundin P. Neurodegeneration and glutamate induced oxidative stress. Prog Brain Res 1998;116:245–63.

Nakao N, Grasbon FEM, Widner H, Brundin P. Antioxidant treatment protects striatal neurons against excitotoxic insults. Neuroscience 1996;73(1):185–200.

Okhawa H, Ohishi N, Yagi K. Assay of lipid peroxides in animals tissue by thiobarbituric acid reaction. Anal Biochem 1979;95:351–8.

Ray PS, Maulik G, Cordis GA, Bertelli AA, Bertelli A, Das DK. The red wine antioxidant resveratrol protects the isolated rat heart from ischemia reperfusion injury. Free Radical Biol Med 1999;27:160–9.

Said SI, Pakbaz H, Berisha HI, Raza S. NMDA receptor activation: critical role in oxidant tissue injury. Free Radical Biol Med 2000;28: 1300–2.

Scultz JB, Henshaw DR, Siwek D, Jenkins BG, Ferrante RJ, Cipolloni PB, Kowall NW, Rosen BR, Beal MF. Involvement of free radicals in excitotoxicity in vivo. J Neurochem 1995;64:2239–47.

Shi MM, Kugelman A, Iwamoto T, Tian L, Forman HJ. Quinone induced oxidative stress elevates glutathione and induces glutamylcysteine synthetase activity in rat lung epithelial L2 cells. J Biol Chem 1994;269: 26512–7.

Sun AY, Chen YM, Kracke MJ, Wixom P, Chen Y. Ethanol induced cell death by lipids peroxidation in PC12 cells. Neurochem Res 1997;22: 1187–92.

Teismann P, Ferger B. Comparison of the noval drug ensacutin with MK-801 on the reduction of hydroxyl radical production in rat striatum after local application of glutamate. Brain Res 2000;857:165–71.